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PREPARATION OF A PURIFIED SORBED TYPE F BOTULISMOTOXOID. REPORT--ETC(U)
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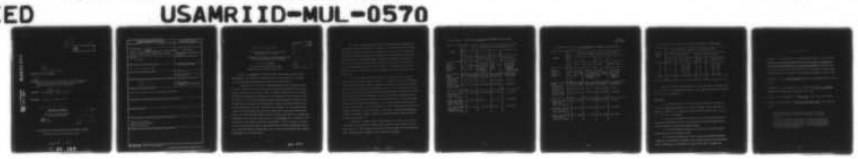

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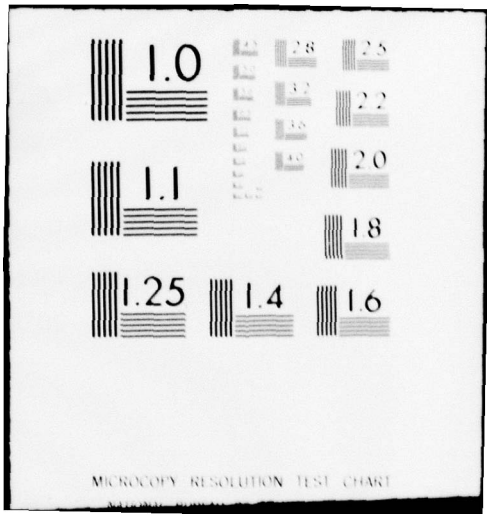
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PREPARATION OF A PURIFIED SORBED TYPE F BOTULISMOTOXOID. REPORT I

(Comparative Assessment of Various Nutrient Media to Obtain Active Type F Botulin)

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(Received by the editors 12 Jan. 1970)

Type F Cl. botulinum was discovered only comparatively recently (Jensen and Hahneman, 1959; Moller and Scheibel, 1960) so that research on the preparation of a botulinic anatoxin of this type has not been extensive.

Researchers have used various different nutrient media to prepare a Type F toxin. Mikhaylova in 1965 obtained toxins containing 3,600-4,000 Dml/ml by culturing strain No. 470 for 3 days at 28°C on Gluzman's medium and also on a casein-hydrolysate medium (longer culturing of 4-5 days produced a drop in toxin activity to 100 Dlm/ml). On the Martin medium, maximum toxin formation was reached on the 7th day (up to 100 Dlm/ml); on liver bouillon, toxicity rose steadily until reaching 700 Dlm/ml by the 7th day. Mikhaylova and Goldshmid (1966) in growing a Type F culture on liver bouillon in flasks with cotton and sausage meat, realized toxicities of 2,000-10,000 Dlm/ml after 7 days of culturing and filtration. Butalova and colleagues (1969) studied 3 strains of Type F Cl. botulinum (Craig, Eklund, No. 470), found that No. 470 showed the greatest toxigenicity. On the 5-6th days of culturing on a casein-fungus medium, its biological activity reached 20,000-40,000 Dml/ml; on a casein-acid medium, 60,000-80,000; and on a fish bone meal hydrolysate, 80,000-100,000 (all tests on white mice).

The present study, aimed at selecting a nutrient medium for preparation of crude and purified botulinic Type F anatoxins, was based on cultures of strain No. 470, using Gluzman's medium, liver bouillon, media with a base of whale meal and casein hydrolysates, Martin type medium with maize extract and maltose, and some other forms of the Martin medium. Culturing was carried out in quarters, with 5 g of cotton at 28°C. The media following sterilization showed a pH of 7.7-8.2, and contained 100-140 mg of amino nitrogen. Beginning with the 4th day, extracts were sterilely removed from the quarters, and Dlm and lt determined on white mice.

The greatest antitoxin-binding capacity was observed with the use of media containing Martin's peptone (2.3-4.2 lt). In liver and whale-maze bouillon, antitoxin-binding activity reached a maximum by the 6-7th day of culturing, amounting to 2.4-2.6 and 0.7-1.4 lt, respectively. Toxicity of the culture on classical Martin's medium and on Martin type medium with maize extract, in every case reached a maximum on the 6th day of culturing; on classical Martin's medium with sausage meat but without chalk, on the 4th day. Toxins obtained on a casein medium showed low activity--210 Dlm/ml and lt under 0.1. Using a whale-maize medium, activity in some series reached $1 \cdot 10^6$, in others $0.05 \cdot 10^6$ on the 7th day, this being explained, apparently, by the instability of the medium. In growing Clostridium on on media with Martin's peptone, lowering of antitoxin-binding capacity was noted on the 7th day (Table 1).

Refrigerator storage (4-6°C) of the culture for a period of 30 days did not alter binding capacity or toxicity, which, actually, even increased in some cases (Table 2). This increase was evidently associated with lysis of the microbe cells and with increased advance of the toxin into the medium.

Toxin Formation by Type F Cl. botulinum with Culturing on Various Media

Medium	No. of series	Result of culturing for various periods (days)			
		4	5	6	7
		Lt/ml	Dml/ml (mil.)	Lt/ml	Dlm/ml (mil.)
Glauzman	8	<1	0.55 ± 0.15	<1	0.65 ± 0.08
Liver	10	1.8 ± 0.45	1.2 ± 0.19	1.8 ± 0.45	1.5 ± 0.24
Whale-maize	10	<1	0.57 ± 0.23	<1	0.57 ± 0.23
Casein	6	<0.1	0.00021 ± 0.00023	<0.1	0.00014 ± 0.00014 [sic]
Martin	18	2.0 ± 0.42	0.96 ± 0.13	2.4 ± 0.25	1.08 ± 0.19
Martin + chalk no sausage	6	1.3 ± 0.54	1.1 ± 0.33	1.8 ± 1.0	1.2 ± 0.33
Martin + saus. no chalk	6	3.0 ± 0	1.5 ± 0	3.3 ± 0.54	1.5 ± 0
Martin + maize extract alone	16	2.1 ± 0.47	1.3 ± 0.23	2.8 ± 0.62	1.6 ± 0.26
Martin type with maize extr. & chalk, no saus.	9	2.8 ± 0.54	1.3 ± 0.28	3.5 ± 0.53	1.4 ± 0.32
Martin type with maize extr. and saus., no chalk	4	2.0 ± 1.8	0.8 ± 0	3.0 ± 1.8	0.9 ± 0.57
Martin type with maize extr. + sausage + chalk	10	3.4 ± 0.41	1.4 ± 0.31	4.0 ± 0	1.9 ± 0.51

TABLE 1
(continued)

Toxin Formation by Type F Cl. botulinum with Culturing on Various Media

Medium	No. of series	Result of culturing for various periods (days)			
		4	5	6	7
		Lt/ml	Dml/ml (mil.)	Lt/ml	Dlm/ml (mil.)
Glauzman	8	1.0 ± 0	1.0 ± 0	1.0 ± 0	1.15 ± 0.31
Liver	10	2.4 ± 0.37	2.1 ± 0.37	2.6 ± 0.54	2.0 ± 0.59
Whale-maize	10	0.7 ± 0.25	0.57 ± 0.23	1.4 ± 0.63	0.42 ± 0.3
Casein	6	<0.1	0.000083 ± 0.000065	<0.1	0.00008 ± 0.000059
Martin	18	3.7 ± 0.53	1.3 ± 0.33	3.0 ± 0.36	1.3 ± 0.36
Martin + chalk no sausage	6	2.3 ± 0.54	1.2 ± 0.33	2.0 ± 0.9	1.27 ± 0.41
Martin + saus. no chalk	6	4.0 ± 0.9	1.5 ± 0	3.3 ± 0.54	1.5 ± 0
Martin + maize extract alone	16	3.2 ± 0.53	1.8 ± 0.34	3.1 ± 0.47	1.5 ± 0.32
Martin type with maize extr. & chalk, no saus.	9	3.7 ± 0.67	1.6 ± 0.48	3.4 ± 0.67	1.6 ± 0.6
Martin type with maize extr. and saus., no chalk	4	3.5 ± 0.89	1.5 ± 0.89	3.0 ± 0	1.5 ± 0.89
Martin type with maize extr. + sausage & chalk	10	4.2 ± 0.33	2.2 ± 0.33	3.6 ± 0.41	1.6 ± 0.31

Activity of Type F Botulinic Toxin Following Culturing and Cold Storage

No. of toxin series	Antitoxin-binding capacity and strength of toxin			
	after 7 days at 37°C		after 30 days at 4-6°C	
	Lt/ml	Dlm/ml (mil.)	Lt/ml	Dlm/ml (mil.)
3	3	$0.8 \cdot 10^6$	3	$1.5 \cdot 10^6$
3	2	$1.2 \cdot 10^6$	2.5	$1.4 \cdot 10^6$
8	2	$0.6 \cdot 10^6$	3	$1.0 \cdot 10^6$
13	4	$0.8 \cdot 10^6$	4	$0.8 \cdot 10^6$
14	2	$0.6 \cdot 10^6$	2.5	$0.8 \cdot 10^6$
M ± m	2.6 ± 1.1	$0.8 \cdot 10^6 \pm 0.28 \cdot 10^6$	3 ± 0.75	$1.1 \cdot 10^6 \pm 0.42 \cdot 10^6$

The most intensive toxin formation, then, was realized by culturing strain No. 470 on media containing Martin's peptone. Toxin activity reached its maximum on the 6th day of culturing.

Conclusions

1. Maximum antitoxin-binding capacity and toxicity of a culture of Type F Cl. botulinum appeared on the 6th day of growth in liver bouillon, on Martin's medium and on a Martin type medium with maize extract (2.3 - 4.2 Lt; $1.2 \cdot 10^6$ - $2.2 \cdot 10^6$ Dlm/ml).

2. No strict proportionality between toxicity and antitoxin-binding capacity was observed, but it was established that maximum antitoxin-binding capacity coincided in time with maximum toxicity.

3. Antitoxin-binding capacity and toxicity did not diminish during 30-day storage of the culture at 4-6°C.

4. Toxins with activity of 3.2-3.7 Lt could be obtained on Martin's medium with maize extract and maltose. This medium, being the least expensive, can be recommended for preparation of highly active Type F preparations.

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PREPARATION OF PURIFIED SORBED BOTULISMOTOXOID, TYPE F. Report I. Comparative Assessment of Various Nutrient Media to Obtain Active Type F Botulin

V. P. Tsarapkin

Comparative assessment of various nutrient media for production of toxin by Cl. botulinum, type F, has shown the greatest antitoxin-binding capacity and toxicity of the culture on the 6th day of cultivation on liver broth, on classic Martin's broth and on a medium of Martin's type with corn extract. The antitoxin binding capacity and toxicity failed to alter after 30-day storage of the culture in a refrigerator at a temperature ranging from 4 to 6° C. A medium of Martin's type with corn extract and maltose can be recommended for obtaining highly active, type F preparations.